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14. ABSTRACT **Hypothesis:** extracellular matrix (ECM) and bone microenvironment cytokines are critical for metastatic breast cancer cells to grow or remain dormant. This hypothesis is being tested using a 3D bioreactor of ECM, derived from osteoblasts (OB). **Aim 1**: determine how modification of the composition and structure of the ECM affects proliferation and dormancy. **1a**. deprive OB of estrogen; **1b**. stress the ECM; **1c**. degrade the ECM with osteoclasts. **Aim 2.** determine how bone-remodeling and inflammatory cytokines affect proliferation and dormancy. **2a**. add/block bone remodeling cytokines, **2b**. add/block OB inflammatory stress response cytokines. Thus far: the remodeling cytokines permit dormant human cells to proliferate in the bioreactor in co-culture with OB. The effect appears to depend on prostaglandin production. Chronic oxidative stress of the ECM with H_2O_2 did not affect cancer cell growth. Howver estrogen deprivation or blocking the estrogen receptor permitted the dormant cells to proliferate. Human breast cancer cells grew better on decellurized matrix or on fixed osteoblasts than on intact matrix indicating that OB produce factor(s) antagonistic to cancer cells.

15. SUBJECT TERMS
Bone metastasis, 3D model, osteoblasts, cytokines, extracellular matrix

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Introduction

Breast cancer often metastasizes to the skeleton. The microenvironment of the bone plays an important role in determining whether the cancer cells grow or become dormant. We hypothesize that the extracellular matrix (ECM) and cytokines of the bone microenvironment are critical in determining the fate of the cancer cells. The study is being carried out in a relevant and innovative 3D model of bone-like tissue derived from osteoblasts. The aims are to determine how modification of the composition and structure of the ECM, and how cytokines and growth factors affect the cancer cells. The composition and structure of the ECM will be modified by deprivation of estrogen, oxidative stress, and by osteoclasts. Cytokines associated with bone remodeling and with inflammation will be added or blocked. We propose to use human primary osteoblasts with human MDA-MB-231 metastatic cells and their metastasis suppressed counterparts, MDA-MB-231BRMS1, and mouse MC3T3-E1 osteoblasts and murine mammary tumor cells D2A1(metastatic) or D2.OR(dormant).

Body

Hypothesis: The ECM and cytokines of the bone microenvironment are critical in determining whether metastatic breast cancer cells will grow or become dormant.

Specific aim: 1: To determine how modification of the ECM composition and structure affects proliferation and dormancy of breast cancer cells

Task 1 a. Deprive osteoblasts of estrogen

Osteoblasts express estrogen receptors. Estrogen is critical for osteoblast development of ECM. Furthermore, breast cancer occurs more commonly in postmenopausal women when estrogen is low. Thus we asked if estrogen withdrawal during osteoblast differentiation would affect the growth of the cancer cells. In order to determine how an ECM formed under estrogen deprivation would affect the growth of cancer cells, murine osteoblasts, MC3T3-E1, were grown for 2 months in the bioreactor with a basal medium of αMEM with 10mM β-glycerophosphate and 50 μg/ml ascorbic acid. Normal FBS (N-FBS) was added in the growth chamber at a concentration of 10% to the control cultures. To simulate estrogen deprivation, 1μm of pure anti-estrogen ICI 182,780 was added to block the effect of estrogen contained in the N-FBS. Other cultures were deprived of estrogen by the use of 10% charcoal stripped FBS (CS-FBS) in place of the normal serum. In a subset of these cultures, 17β estradiol was added at 100pg/ml. We had found (reported in the 2013 progress report) that low estrogen in the cultures did not affect osteoblast differentiation, i.e. alkaline phosphatase production, but did suppress mineralization (von Kossa stain). Dormant/proliferating cancer cells were added at 2000 cells/cm^2 . Cultures were imaged by confocal microscopy. Proliferation was quantified with Image J analysis (Figure 1).

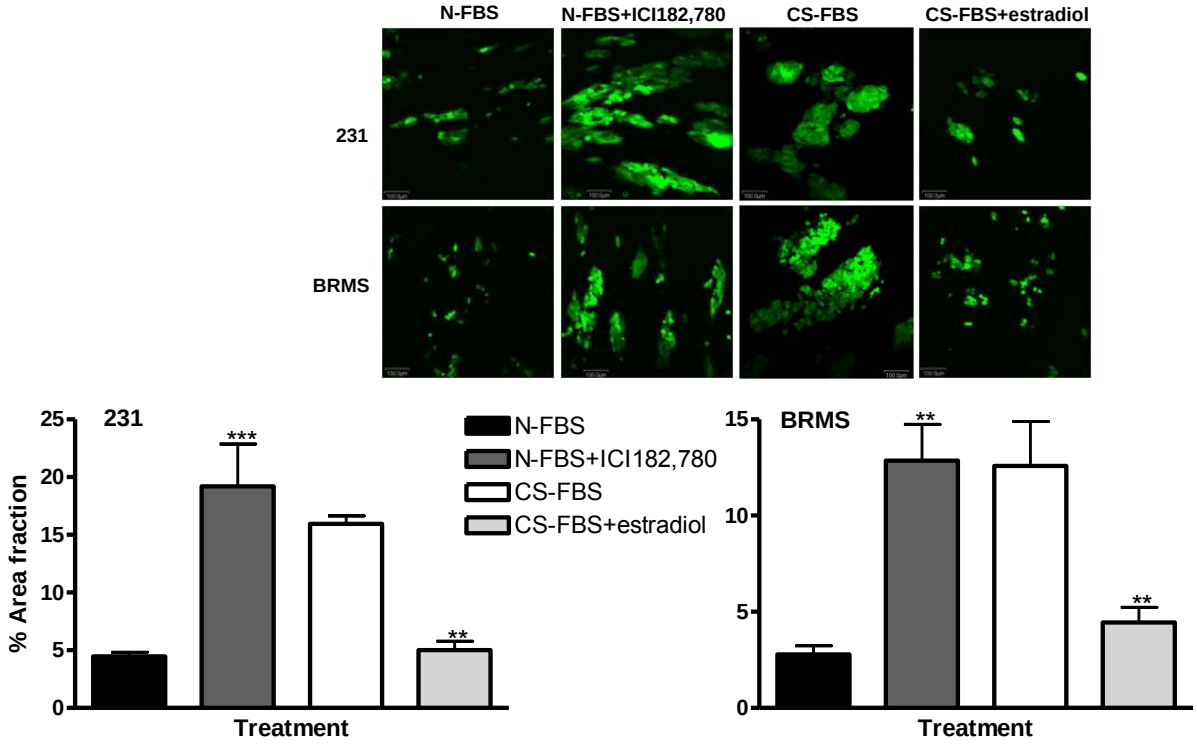


Figure 1. **Estrogen deprivation increased the growth and changed the morphology of MD-MB-231 and MDA-MB-231BRMS1 cancer cells.** Osteoblasts were cultured for two months in αMEM with 10mM β-glycerophosphate and 50 μg/ml ascorbic acid and 10% normal (N) FBS with and without the estrogen receptor antagonist, ICI182,780 (1μM). Osteoblasts were also cultured in the same medium with charcoal stripped (CS) FBS with and without 17-β estradiol (100pg/mL). GFP-expressing metastatic MDA-MB-231 or metastasis suppressed MDA-MB-231BRMS1 cancer cells, were added to each culture; estrogen conditions were maintained after addition of cancer cells. Images were collected daily for 5 days. Shown are representative images and quantitative data from day 5 (n=3). In both 231 and BRMS cells, growth increase occurred with lowered estrogen, i.e. addition of ICI182,780 or with the use of charcoal stripped serum. In cultures containing supplemental estradiol with CS-FBS, the growth and morphology reverted back to normal. Note that both cancer cell lines are estrogen receptor negative. **$p<0.05$; ***$p<0.001$

hypothesize that the breast cancer-osteoblast inflammatory response may be exacerbated with diminished estrogen (Carlsten, 2005).

Task 1. b. Treat the ECM with H_2O_2 prior to the addition of cancer cells.
As reported in the 2013 progress report, we found no significant difference in breast cancer cell growth after treatment of the ECM with H_2O_2. We varied concentrations and times of addition but saw no changes in the cancer cell growth. We plan to try a cyanide containing analog (Tivari et al., 2014).

Task 1.c. Incubate the osteoblast tissue with osteoclasts to partially degrade the matrix.
Pre-osteoclasts must be isolated from the bone marrow and differentiated to active osteoclasts. We have had limited trials of addition of osteoclasts to the bioreactor. We will continue this aspect of the project.

Characterization of the collagen matrix
In order to characterize the collagen in the matrix we had submitted samples of cultures of MC3T3-E1 grown in the bioreactor for two months, to Dr. Patricia Keeley at the University of Wisconsin-Madison. She has carried out second harmonica generation analysis of collagen applied directly to substrates (Gehler et al., 2013). Unfortunately she was unable to resolve the collagen to the desired degree of resolution for second harmonic generation studies. We believe this problem was due to the Surlyn substrate that the cells are grown on in the bioreactor. However, we had read of a novel and recently discovered collagen binding protein, CNA35, isolated from S. aureus, that could be labeled with a fluorescent probe (Zong et al., 2005). This molecule can be used with live or fixed cultures. We obtained CNA35 from Dr.Magnus Hook, Texas A&M. We conjugated it with either Alexa Fluor 568 or 488. The conjugated protein was used in cultures at 0.05 μM. We were successful in labeling collagen fibrils in 2D and 3D cultures (Figure 2). MDA-MB-231 GFP expressing cancer cells were added to cultures of MC3T3-E1 at 4000 cells/cm^2. After 3 days of co-culture the cells were stained with CNA35-Alexa Fluor 568 and imaged by confocal microscopy. The collagen arrangements of the matrices were different for the same cells grown under the two different conditions (compare Figure 2A with Figure 2B).

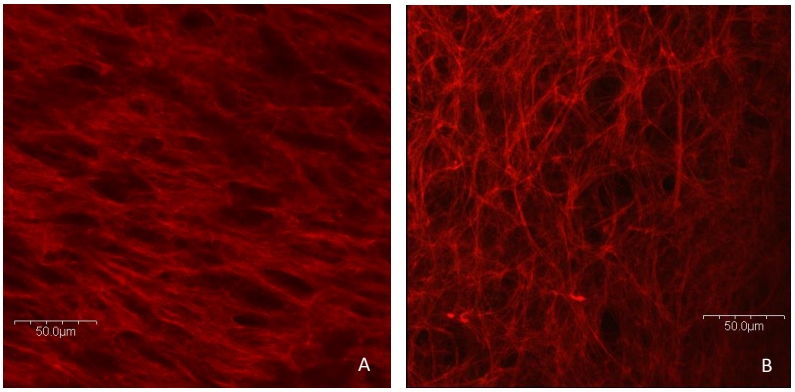


Figure 2. MC3T3-E1 osteoblast matrix labeled with CNA35-Alexafluor 568 in 2D (A) or 3D (B) cultures. MC3T3-E1 were cultured in tissue culture plastic plates (A) for 3 weeks or in the bioreactor (B) for 4 weeks with αMEM, 1% β-glycerol phosphate and 50 µg/ml ascorbic acid. CNA35 was added to fixed cultures at 0.05 µM. Images were taken with confocal microscopy.

GFP-expressing MDA-MB-231 cells were added to 2D cultures and imaged 3 days later (Figure 3). The cancer cells aligned themselves within and along the matrix and appeared to remodel the neighboring fibers.

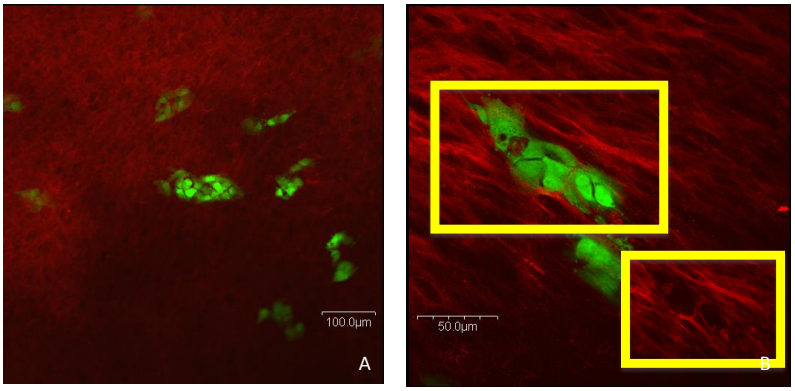


Figure 3. Remodeling of osteoblast-produced ECM by MDA-MB-231 cells in 2D cultures. MC3T3-E1 osteoblasts were cultured for 3 weeks before addition of MDA-MB-231-GFP (4000 cells/cm^2). Three days later the cultures were fixed, stained and imaged with confocal microscopy. A, 20X; B, 60X magnification. Note the changes in the ECM close to the cancer cells, and that some cancer cells are buried in the ECM.

We also tested the MDA-MB-231-GFP cells in the bioreactor (Figure 4). The MC3T3-E1 had been grown for 4 weeks prior to the addition of cancer cells. They were imaged as in Figure 4 . The matrix in the bioreactor appears to be thicker than in the culture dish. Thus it is more difficult to see the changes in the individual collagen fibers.

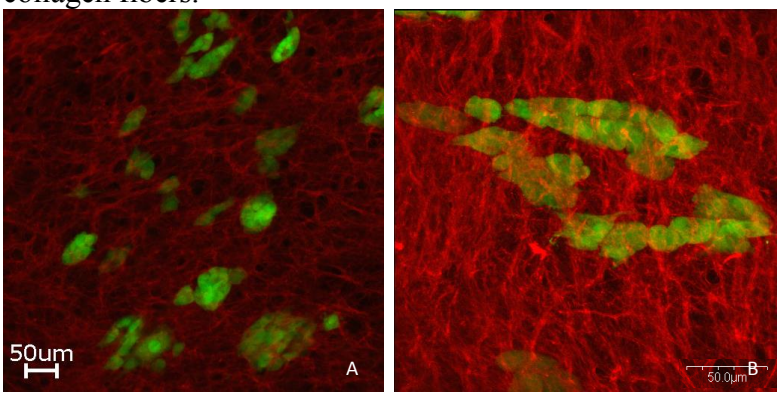


Figure 4. Metastatic MDA-MB-231 cells invade the ECM in the bioreactor. The cancer cells were added to 4 week cultures of MC3T3-E1 cells. After 3 days the cultures were fixed, stained with CNA35 conjugated to Alexa Fluor 568 and imaged with confocal microscopy at 20X (A) or 60X (B). Note how the cancer cells are imbedded beneath the matrix.

In another set of bioreactor cultures we tested the metastasis suppressed variant of MDA-MB-231, MDA-MB-231-BRMS1 cells (Figure 5).

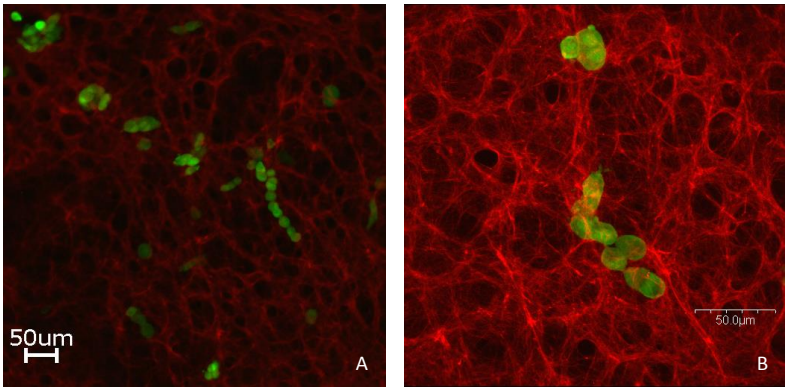


Figure 5. MDA-MB-231BRMS1 cells were less invasive in the ECM. MC3T3-E1 cells were grown in the bioreactor for 4 weeks before the addition of the metastasis suppressed cancer cells. After 3 days the cultures were fixed, stained with CNA35 Alexa-568 and imaged with confocal microscopy at 20X (A) or 60X (B). In comparison to the metastatic MDA-MB-231 cells the BRMS cells remained above the matrix.

The CNA35 has provided us a tool to begin to investigate the interaction of the cancer cells with the ECM and to ask how the ECM affects the growth of the cancer cells. We will use the CNA35 to examine the matrix after deprivation of estrogen and after treatment with oxidizing agents or the addition of osteoclasts.

Atomic Force Microscopy

Atomic force microscopy will provide a wealth of information to characterize the bone-like matrix. The Bruker ICON located in the Materials Research Institute, Penn State University Park, is capable of performing peak force tapping analysis which is particularly well suited for atomic force microscopy of delicate biological samples. The instrument has expanded capability to acquire quantitative information for such matrix characteristics as topography, elasticity, stiffness and adhesion. A member of our research team has attended a two day workshop on the principles and operation of the atomic force instrumentation. We plan to characterize and compare samples of the native-made osteoblast matrix with and without cancer cells.

Aim 2: To determine how bone remodeling and inflammatory cytokines in the microenvironment affect proliferation and dormancy of cancer cells.

Task 2a: Cocktails of cytokines associated with bone remodeling were tested for the effects on growth/dormancy of cancer cells.

Bioreactor MC3T3-E1/Human Breast Cancer Cell Model

In the 2013 progress report, we reported that MDA-MB-231BRMS1 breast cancer cells entered a dormant state when cultured in a bioreactor with differentiated MC3T3-E1 murine osteoblasts. The osteoblasts at two months of culture had formed a thick collagen matrix (Mastro and Vogler, 2009). When a cocktail of bone remodeling cytokines (TNFα, IL-1β and IL-6) were added, the cells proliferated and exhibited a dramatic change in morphology. To demonstrate that the cytokines were directly responsible shift to the proliferative state, we carried out experiments in which the cytokines were inhibited by neutralizing antibodies. MC3T3-E1 cells were grown and differentiated in the bioreactor for two months, establishing a mature osteoblast population with a native collagenous matrix. The human breast cancer cell lines were added (4000 cells/cm^2) to the bottom chamber of the bioreactor cultures; 15 minutes later, a limited panel of bone remodeling cytokines (TNFα (5ng/mL), IL-1β (10ng/mL), and IL-6 (10ng/mL)) was added. Neutralizing antibodies to each of the cytokines were added to the cytokine cocktail in 600-2000 fold excess. Cytokines and antibodies were purchased from R&D Systems, Minneapolis, MN. Cultures were photographed daily by confocal microscopy for morphological

and proliferative changes in the cancer cells. On the final day of culture, bioreactors were dismantled and the culture membranes fixed in 4% paraformaldehyde. Culture supernatants were frozen for later cytokine analysis. Image J analysis was conducted on collected confocal images to determine percent area fraction. Results are the average of 3 images collected at each time point. Statistical analysis was performed in GraphPad Prizm using 2-way ANOVA.

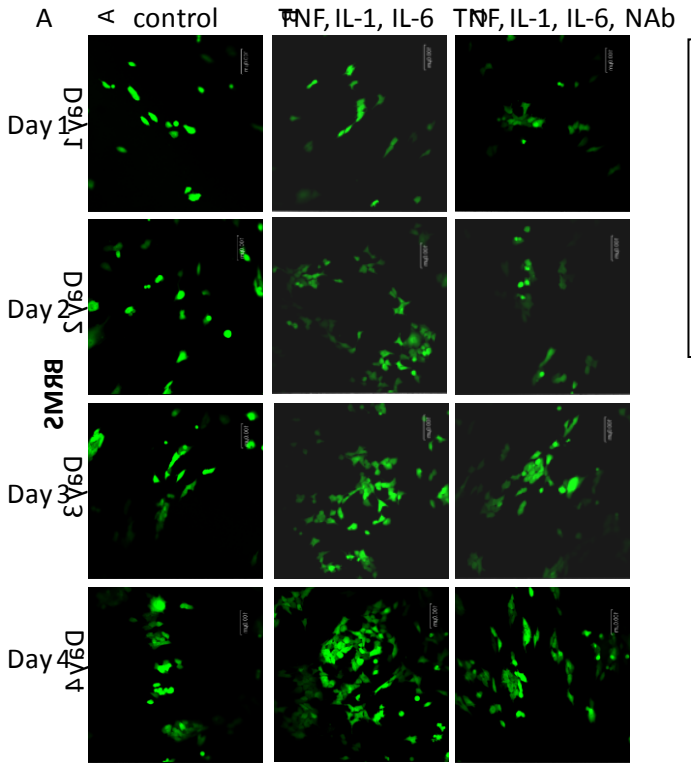
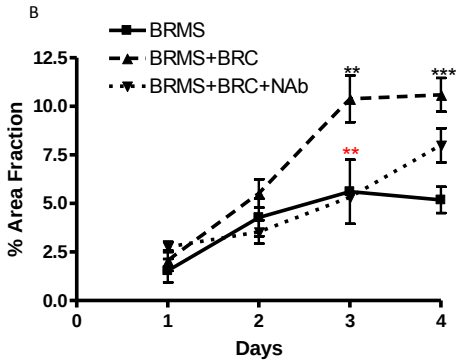


Figure 6. The effects of BRC on BRMS cells were mitigated by neutralizing antibodies. A) TNFα, IL-1β and IL-6 were added to the 3D osteoblast culture along with neutralizing antibodies. Cultures were imaged daily for 4 days. Shown are representative images for each time. B) Area fraction quantitation was performed on images (n=3) using ImageJ. **p<0.01 ; ***p<0.001



The presence of the neutralizing antibodies to the bone remodeling cytokines blocked the proliferative and morphological changes elicited in the MDA-MB-231BRMS1 cells (Figure 6A). However, after three days in culture this effect diminished (Figure 6B). It is possible that the antibodies were degraded to the point that they were no longer effective.

Next, we set out to investigate possible mechanisms for the "awakening" of the dormant BRMS1 cells by the bone remodeling cytokines. A search of scientific literature revealed a strong correlation between cyclooxygenase-2 (COX-2) and its downstream product, prostaglandin E2 (PGE) in the tumorgenesis and invasion of breast cancer cells (Mitchell et al., 2010). Cultures were established as previously described. The COX-2 inhibitor, indomethacin (Sigma-Aldrich, St. Louis, MO) was added to select cultures at a concentration of 50μM. Cultures were photographed daily by confocal microscopy for morphological and proliferative changes in the cancer cells. On the final day of culture, bioreactors were dismantled and the culture membranes fixed in 4% paraformaldehyde. Culture supernatants were frozen for later cytokine analysis. Image J analysis was conducted on collected confocal images to determine percent area fraction. Results are the average of 3 images collected at each time point. Statistical analysis was performed in GraphPad Prizm using 2-way ANOVA.

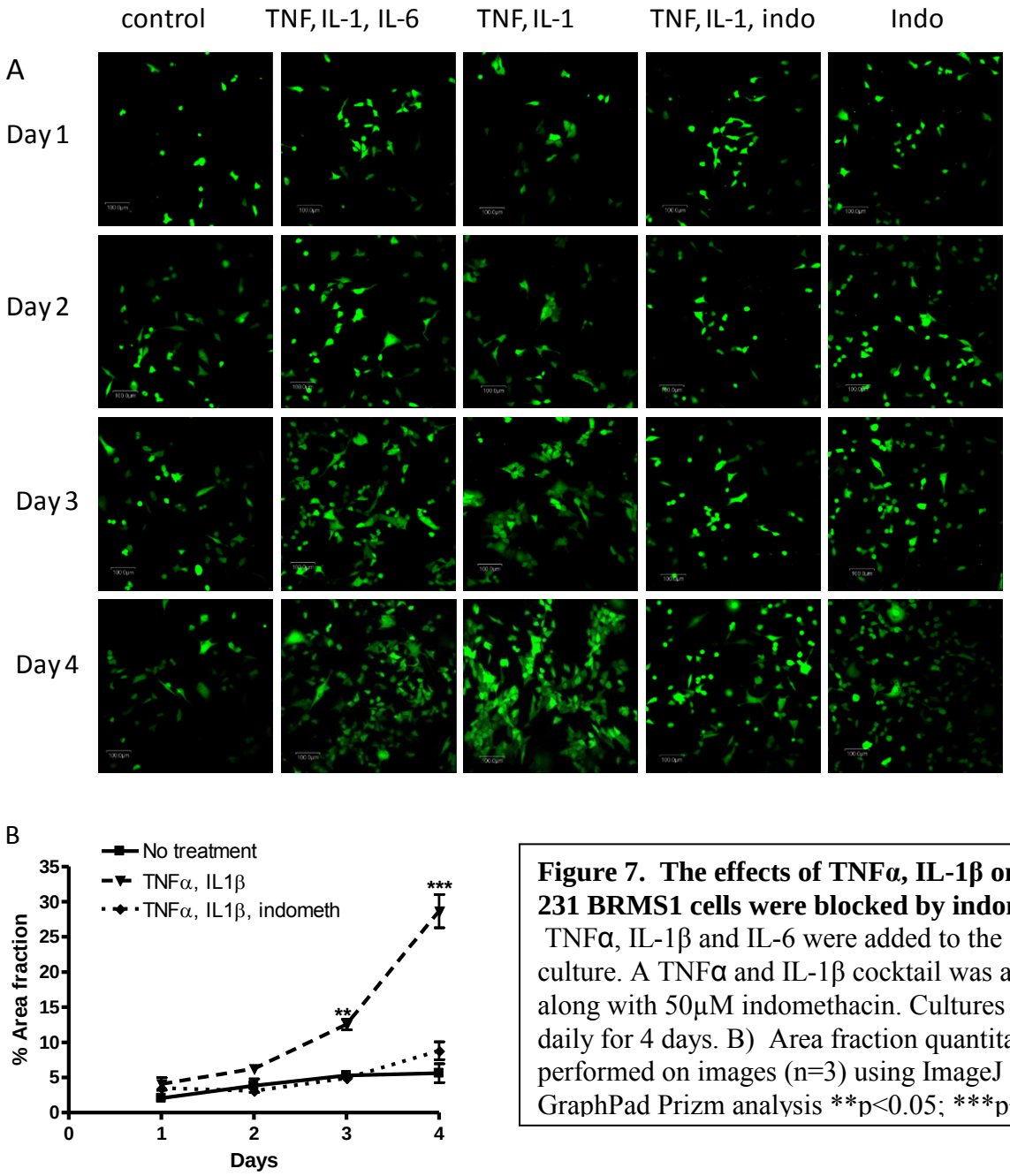


Figure 7. The effects of TNFα, IL-1β on MDA-MB-231 BRMS1 cells were blocked by indomethacin. A) TNFα, IL-1β and IL-6 were added to the 3D osteoblast culture. A TNFα and IL-1β cocktail was also tested along with 50μM indomethacin. Cultures were imaged daily for 4 days. B) Area fraction quantitation was performed on images (n=3) using ImageJ and GraphPad Prizm analysis **p<0.05; ***p<0.001

From this experiment, we determined that IL-6 was not required for the BRMS1 cells to break dormancy; TNFα and IL-1β were sufficient to cause the proliferative and morphological changes. The addition of 50μM indomethacin caused the cells to maintain their dormant state, even in the presence of the bone remodeling cytokines (Figures 7A and 7B).

In light of these findings, we decided to target molecules downstream of COX-2 in the arachidonic acid cascade, namely PGE2. The prostaglandin receptor inhibitor AH6809 (from Cayman Chemical, Ann Arbor, MI) was added to the cultures at a concentration of 50μM simultaneously with the TNFα and IL-1β. Cultures were maintained and images collected daily for 4 days.

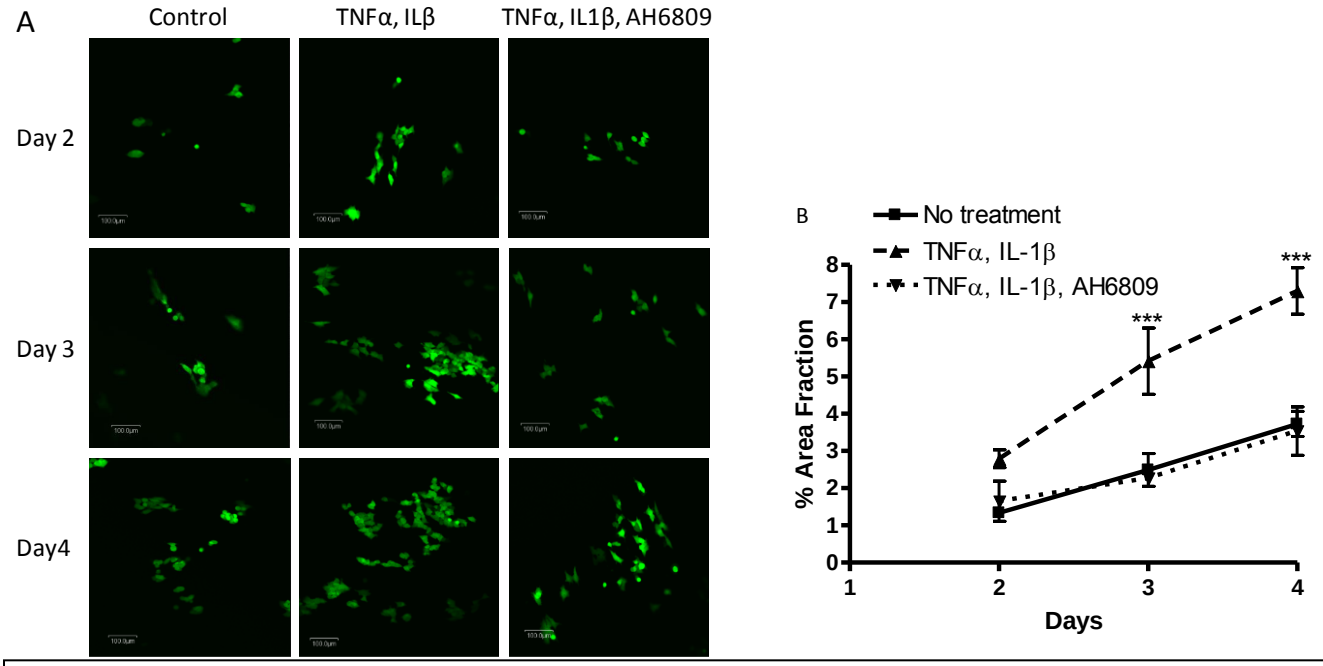


Figure 8. The effects of TNFα, IL-1β on MDA-MB-231BRMS1 cells were blocked by the PGE2 receptor inhibitor, AH6809. TNFα and IL-1β were added to the 3D osteoblast culture along with 50μM AH6809. Cultures were imaged daily for 4 days. A) Representative images of live bioreactor cultures captured by confocal microscopy. B) Area fraction quantitation was performed on images (n=3) using ImageJ; graph and statistical analysis was performed on GraphPad Prizm using 2-way ANOVA. ***p<0.001

The prostaglandin receptor inhibitor AH6809 had a marked inhibitory effect on the proliferation and morphology changes induced by the bone remodeling cytokines TNFα and IL-1β (Figure 8A). The area fraction occupied by cancer cells was reduced to the same level as the co-culture without additional cytokines (Figure 8B). These data suggest that PGE2 plays a key role in the switch of a breast cancer cell from a dormant to an active state. In order to investigate this link, we assayed the reserved supernatants from the bioreactor cultures for levels of PGE2 using an EIA based kit (GE Healthcare, Piscataway, NJ). Assays were performed according to the manufacturer's protocol with recommended modifications to allow for the high protein content of the bioreactor supernatants.

Treatment	PGE2 (pg/mL)
None	337
TNF, IL-1, IL-6	9351
TNF, IL-1	1909
TNF, IL-1, Indo	376

Treatment	PGE2 (pg/mL)
None	260
TNF, IL-1	35107
TNF, IL-1, NAb	348
TNF, IL-1, AH6809	32383

Figure 9. PGE2 levels are elevated by the bone remodeling cytokines, but reduced by addition of indomethacin or neutralizing antibodies. The results of two separate experiments are summarized in the tables above. In both cases, PGE2 levels are elevated well above the level of untreated cultures while levels are decreased in cultures with added indomethacin or neutralizing antibodies. The PGE2 receptor inhibitor AH6809 did not lower the level of PGE2 induced by the bone remodeling cytokines.

The tables above represent two separate experiments. In both cases, the levels of PGE2 were substantially elevated when TNFα and IL-1β were present. That elevation was mitigated by the addition of the COX-2 inhibitor, indomethacin which prevented the downstream production of PGE2 in the arachidonic acid metabolic pathway. While the cultures containing the cytokines plus the PGE2 receptor inhibitor, AH6809 produced high levels of PGE2, the cytokine-associated effects on growth and morphology of the BRMS1 cells were halted by the inability of the prostaglandin to bind to the cell receptor. These data indicated that the bone remodeling cytokines TNFα and IL-1β caused dormant cells in a bone microenvironment to proliferate via increased production of prostaglandin E2 signaling.

Bioreactor culture of normal human osteoblasts (NHOst) and human breast cancer cells

As stated in the 2013 progress report, we were successful in establishing bioreactor cultures of the normal human osteoblasts purchased from Lonza (Walkersville, MD). These cultures produced a rich collagenous matrix and mineralized after one month in culture. However, when MDA-MB-231BRMS1 breast cancer cells were co-cultured with these osteoblasts in the bioreactor, they failed to show the dormancy model seen with the murine osteoblast/ human cancer cell system.

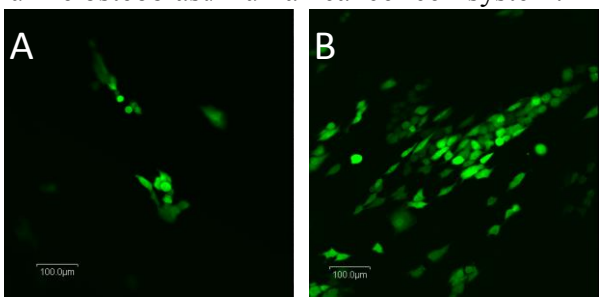


Figure 10. MDA-MB-231BRMS1 breast cancer cells co-cultured for 3 days with MC3T3-E1 murine and NHOst human osteoblasts. A) BRMS1 cancer cells grown on a 2 month old bioreactor culture of MC3T3-E1 osteoblasts. B) BRMS1 cells grown on a 1 month old bioreactor culture of NHOst osteoblasts. Note marked differences in morphology and growth.

The MDA-MB-231BRMS cells clearly proliferated and assumed a morphology similar to the aggressive, non-suppressed MDA-MB-231 cells when grown in culture with human osteoblasts (Figure 10B). We speculate that the NHOst osteoblasts may be secreting a cytokine or growth factor that is not produced by the MC3T3-E1 osteoblasts that could be triggering proliferation of the cancer cells. Since dormancy could not be established from the outset, this cell pairing proved to be unsuitable as a dormancy model. However, comparison of the two cultures, MC3T3-E1 and NHOst may prove valuable.

The well characterized non-metastatic breast cancer cell line MCF-7 has been used by other researchers as a dormancy model (Tivari et al., 2014). This cell line has been shown to enter a dormancy state when cultured at low density with basic fibroblast growth factor (bFGF). For these experiments, NHOst cells were seeded in the bioreactor chamber at a density of 10^4 cells/cm^2. The lower chamber contained 5mL of Lonza OB differentiation media supplemented with 15% fetal bovine serum (FBS). The upper chamber contained the same medium minus the FBS. The upper media reservoir was exchanged after two weeks of culture. One day prior to seeding the cancer cells in the reactor, the medium in the upper chamber was removed and replaced with basal osteoblast growth medium in order to dialyze out the hydrocortisone which is a component of the differentiation media. Prior to seeding the cancer cells, the upper chamber medium was again removed and replaced with growth medium. The GFP-labeled MCF-7 cells were seeded at a density of 2000 cells/cm^{2}together with bFGF (10ng/mL). One hour later, a cocktail of bone remodeling cytokines (BRC) was added to some reactors. This mixture consisted of TNFα (5ng/mL), IL-1β (10ng/mL), IL-8 (0.5ng/mL), IL-6 (10ng/mL), and MCP-1 (2ng/mL). To test if the COX-2/PGE2 inhibition would suppress the effects of the cytokines on dormancy, some reactors received 50µM indomethacin or 50µM AH6809 (PGE2 receptor inhibitor. Cultures were maintained for 3 days; images were captured by confocal microscopy and analyzed by Image J.

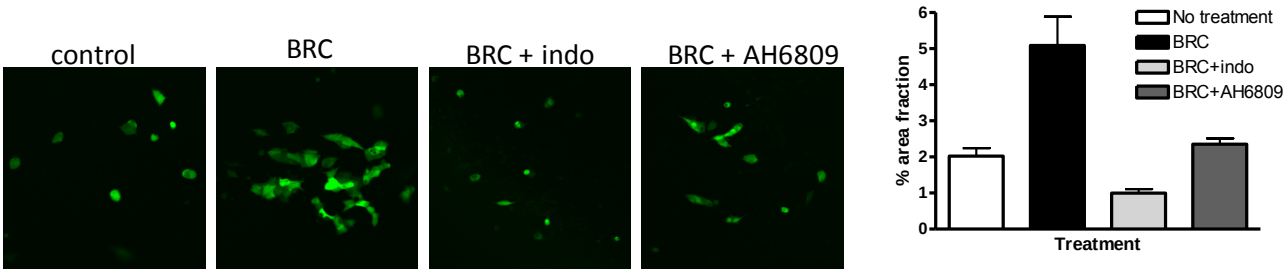


Figure 11. Bone remodeling cytokines increased the growth and altered morphology of MCF-7 cells in a dormancy model via a COX-2/PGE2 pathway. MCF-7 breast cancer cells were co-cultured with 1 month old human osteoblasts (NHOst) in a bioreactor system. A mixture of bone remodeling cytokines (BRC) was added in addition to 50μM indomethacin or 50μM PGE2 receptor, AH6809. Shown are representative images on day 3. Statistical analysis is based on Image J area quantitation for (n=6) images obtained on day 3

The MCF-7 human breast cancer cells proved to be an excellent model to study cancer cell dormancy in a bone-like microenvironment. With the addition of basic FGF, the cells became dormant; the addition of bone remodeling cytokines caused a marked morphological change accompanied by an increase in proliferation. These effects were mitigated by the addition of indomethacin and, to a lesser extent, AH6809. These findings support the evidence seen in the previous experiment for the important role of bone remodeling cytokines in the awakening of dormant cancer cells.

Alternative model for breast cancer cell dormancy in bone

The bone and its marrow compartment contain a wide variety of cell types in addition to the osteoblasts. The marrow compartment is said to consist of an outer endosteal niche which is closest to the bone and a vascular niche which occupies the center of the compartment. As the name implies, the endosteal niche is home to osteoblasts and hematopoietic cells while the vascular niche hosts endothelial and stromal cells. Each niche has its own array of secreted molecules, matrix composition and intercellular crosstalk networks which are likely to affect disseminated cancer cells in different ways. Perhaps the vascular niche could be considered as a hospitable environment for breast cancer cells to remain dormant. What would happen if those dormant cells were suddenly exposed to bone remodeling cytokines, a likely occurrence after a bone break? To investigate this hypothesis, we seeded bioreactors with bone marrow endothelial cells at a density of 10,000 cells/cm^2 for 10 days in DMEM, 10% FBS. MDA-MB-231 and MDA-MB-231BRMS1 breast cancer cells were added at a density of 2000 cells/cm^2 and the culture incubated for 24 hours. Bone remodeling cytokines (TNFα (5ng/mL), IL-1β (10ng/mL), IL-8 (0.5ng/mL), IL-6 (10ng/mL), and MCP-1 (2ng/mL)) were added and cultures photographed by confocal microscopy for 3 days.

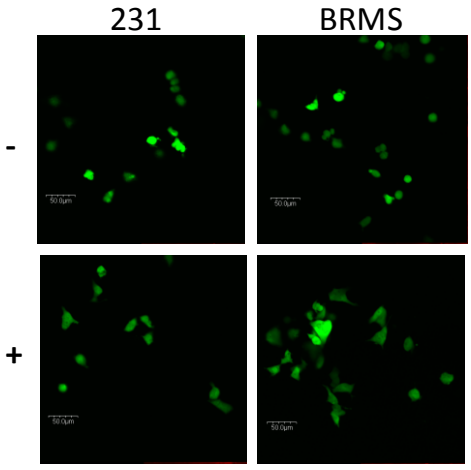


Figure 12. MDA-MB-231 and MDA-MB-231BRMS1 co-cultured in bioreactor with bone marrow endothelial cells with (+) and without (-) bone remodeling cytokines. The 231 and BRMS cells did not appear to proliferate readily on a layer of BMECs. They assumed a rounded morphology. With the addition of bone remodeling cytokines, the morphology changed to a more stellate form and proliferation increased. Images shown were captured 3 days after addition of the cytokines.

The use of bone marrow endothelial cells to represent the vascular niche has provided new avenues to explore with regard to dormancy and growth of breast cancer cells in the bone.

Key Research Accomplishments

- Determined that bone remodeling cytokines that break dormancy of MDA-MB-231BRMS1 through the production of prostaglandins, specifically PGE2. The break can be prevented with indomethacin or an inhibitor of the PGE2 receptor.
- Determine that growth of MC3T3E-1 cells in the absence of serum allows both MDA-MB-231 and MDA-MB-231BRMS1to proliferate more. Addition of estradiol to the culture reverses the effect.
- Developed the use of a collagen binding protein, CNA35, in our laboratory to use to characterize the ECM after various treatments.

Reportable outcomes

Abstracts

Chen, Y.C, **Andrea M. Mastro,** Donna M Sosnoski, Robert J. Norgard, Cassidy D. Grove, Erwin A. Vogler. 2014. Dormancy and growth of metastatic breast cancer cells in a bone-like environment. Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 April 5-9; San Diego, CA.

Pursnani, Richa. The Role of Osteoclasts in the Invasion of Bone by Breast Cancer Cells. 2014 Undergraduate Exhibition Poster Sessions, April 9, Penn State University, University Park, PA. (Honorable Mention, Life Sciences).

Norgard, Robert. The Effects of Breast Cancer on Collagen Production and Architecture on an in vitro Bone-Like Matrix. 2014 Undergraduate Exhibition Poster Sessions, April 9, Penn State University, University Park, PA. (Second prize, Life Sciences)

Theses

Richa Pursnani. An investigation of the Chemotactic Role of Osteoclasts in Breast Cancer Metastasis in Bone. Senior Honors Thesis, Schreyer Honors College, Penn State University, April 7, 2014.

Emily Rutan Impact of Osteoblast-Derived Bone Remodeling cytokines on Metastatic Breast Cancer Cell Dormancy. Senior Honors Thesis, Schreyer Honors College, Penn State University, April 7, 2014

Funding applied for

Robert Norgard and Richa Pursani applied from undergraduate research funds from the Eberly College of Science at Penn State. They were awarded $1000 towards the execution of the projects described in the abstracts.

Research opportunities due to this award

Robert (Bobby) Norgard, a graduating microbiology major, has been working on this project. He made very helpful suggestions for modification of the bioreactor device. He worked on the application of CNA35, a collagen binding protein, to the study the architecture of the college in the bioreactors.
I was able to write strong letters due to his work in the laboratory. He was accepted into 5 prestigious graduate programs. He has accepted the award at University of Pennsylvania.

Two other undergraduate, Emily Rutan and Richa Pursnani, have worked on aspects of this project. Emily has been accepted into Medical School at the University of Massachusetts, and Richa into Medical School at Temple University.

Conclusion

We created a bone like environment in a 3D bioreactor using osteoblasts that had differentiated for several months to lay down a rich collagen matrix. We used this culture system to test the role of the matrix and cytokines in maintaining or breaking dormancy of breast cancer cells. We found that both cytokines and the extracellular matrix play a role in the growth of metastatic and metastasis suppressed breast cancer cells in this bone-like microenvironment. Of the remodeling cytokines, IL-1β, TNFα, are sufficient to stimulate the growth of metastasis-suppressed MDA-MB-231 cells co-cultured in a 3D bioreactor with the cultured osteoblasts. The presence of these cytokines leads to production of prostaglandins. We could prevent the break in dormancy by the addition of indomethacin or an inhibitor of the prostaglandin receptor to PGE2. We also found that a matrix formed in the absence of estrogen increased the growth of both metastatic and metastasis suppressed breast cancer cell. Addition of estradiol to the estrogen deprived cultures had an opposite effect. We also found the MCF-7, a non-metasatic breast cancer line, also grew in the presence of bone remodeling cytokines when cultured with human primary osteoblasts. Finally, we adapted to our laboratory a collagen binding protein, CNA35, to study the structure of the collagen under various conditions. We also plan to try Atomic Force Microscopy to evaluate the properties of the matrix.

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